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A RAPID METHOD OF DETERMINING THE PRESENCE AND TYPE OF BOTULINUS TOXIN IN CONTAMINATED FOODS *

PAUL F. ORR

From the Department of Preventive Medicine and Hygiene, Harvard University Medical School, Boston

The importance of an early diagnosis of botulism like that of tetanus and diphtheria is emphasized by the fact that the benefits of antitoxin depend on early administration. In the case of botulism this is confirmed by clinical reports and experiments. Thus, Forssman [1] concludes from his work on guinea-pigs and rabbits that botulinus antitoxin, to have marked therapeutic effects, must be given before the appearance of respiratory symptoms. Kempner,[2] Dickson and Howitt [3] and others, however, have shown that it is possible to save guinea-pigs by the injection of botulinus antitoxin as long as 24 hours after the injection or feeding of a fatal dose of toxin (fatal in 48 hours), even though definite symptoms of intoxication were manifest.

There are at least two distinct types of botulinus toxin, known as A and B. The antitoxin prepared against each type is specific for the homologous toxin and will not protect against the heterologous toxin. These facts clearly indicate the importance of using both types of antitoxin provided the type specific for the toxin causing the poisoning is not known.

The problem at hand then includes, in addition to an early diagnosis, the type determination of the infecting organism.

Graham and Schwarze [4] suggest the feeding of the responsible food to chickens as a preliminary method of determining the type strain of B. botulinus. They found that type A is fatal when fed to mature

Received for publication, May 16, 1921.

* In accordance with the nomenclature recommended by the Society of American Bacteriologists (J. Bacteriol., 1920, 5, p. 191), Bacillus botulinus becomes Clostridium botulinum (van Ermengen).

* This is part of an extensive investigation on food poisoning, with special reference to botulism, that is being done under the auspices of the Advisory Committee on the Toxicity of Preserved Foods of the National Research Council under a grant from the National Canners' Association.

[1] Centralbl. f. Bakteriol., I. O., 1901, 29, p. 541.

[2] Ztschr. f. Hyg. u. Infectionskrankh., 1897, 26, p. 481.

[3] Jour. Am. Med. Assn., 1920, 74, p. 718.

[4] Jour. Infect. Dis., 1921, 28, p. 317.

chickens while type B does not produce illness in chickens even when fed in liberal amounts. In results to be published elsewhere I have found that it is possible to produce botulism in chickens by feeding foods infected with type B as well as with type A. At least one outbreak of limberneck in chickens reported by Dickson [3, 5] was shown to be caused by B. botulinus, type B. This outbreak occurred at Berkeley, Calif., during 1918. From these observations it is apparent that the method of Graham and Schwarze of typing B. botulinus by the feeding of chickens cannot be accepted.

During the course of work in this laboratory I have found that the intraperitoneal injection in white mice of at least 100 M L D of botulinus toxin produced symptoms of botulism usually in from 2 to 4 hours and death follows the appearance of symptoms within from 1 to 2 hours. It occurred to me that advantage could be taken of this observa-

TABLE 1

TOXIN 23, TYPE A: VARIATION OF LENGTH OF IUCUBATION PERIOD WITH VARIABLE AMOUNTS OF TOXIN; M. L. D.—0.0001 c c

Weight of Mouse in Grams	Amount Injected in C c	Number M L D	Results
15	0.3	3,000	Mouse died within 2 hours and 10 minutes
15	0.1	1,000	Mouse died within 4 hours and 25 minutes
14	0.03	300	Mouse died within 4 hours and 25 minutes
16	0.01	100	Mouse died within 6 hours and 30 minutes
14	0.003	30	Mouse died within 7 hours
15	0.0003	3	Mouse died within 24 hours

tion to determine the presence and also the type of toxin within a few hours. The usefulness of the test, however, is dependent on the possession of the infected food and also on the potency of the toxin in this food. The weaker the toxin the longer the time necessary to obtain the results, owing to the longer period of incubation in mice. Table 1 shows the variation in the length of the incubation period when injecting mice with variable amounts of toxin.

From these results it is evident that the period of incubation in botulism is dependent on the amount of toxin. This corresponds to similar observations on tetanus and diphtheria toxins. It also follows that some idea of the potency of the toxin in infected foods can be obtained from the length of the incubation period in mice which have been inoculated intraperitoneally.

The method of type determination consists in the intraperitoneal injection in each of a number of white mice of about 0.5 c c of the

[5] Jour. Am. Med. Assn., 1918, 71, p. 518.

filtrate of the infected food, some of the mice having been previously injected with type A antitoxin and some with type B antitoxin. If previously immunized mice are not available, it is just as good to mix some of the suspected toxic filtrate with type A antitoxin and some with type B antitoxin, and then inject the mixture into the mice intraperitoneally. Mice of 15 to 20 gm. of weight tolerate 1 c c of non-irritating fluid in the peritoneal cavity very well. If the food contains the toxin of B. botulinus type A the mice receiving no antitoxin and those receiving type B antitoxin will die, while those receiving type A antitoxin will survive. On the other hand, if the food contains toxin of the type B organism only those receiving type B antiserum will live. In this way both the presence and the type of toxin may be determined in from 4 to 6 hours.

TABLE 2

Weight of Mouse in Grams	Amount of Filtrate Injected Intraperitoneally in C c	Amount of Antitoxin Injected Intraperitoneally		Results
		Type A	Type B	
10	0.1	.....	.....	Mouse died in 2 hours
25	0.1	.....	.....	Mouse died in 7 hours
12	0.004	.....	.....	Mouse died in 74 hours
11	0.001	.....	.....	Mouse survived
11	0.1	.....	0.1 c c	Mouse died in 4 hours
12	0.01	.....	0.1 c c	Mouse died in 20 hours
14	0.1	0.1 c c	.....	Mouse survived
12	0.01	0.1 c c	.....	Mouse survived

The basis of this method is not original, however, the extremely short incubation period of the toxin when large amounts are injected intraperitoneally in mice has not been previously reported, and it is the application of this observation in the method which seems to make it worth while to report.

From foods such as string beans, spinach, asparagus and olives it is possible to use the accompanying liquor as the material for injection, while with other foods, such as meat, or when liquor is not available, the food should be thoroughly triturated with a small amount of water or salt solution and the resulting liquid used for injection. It is preferable to filter the liquor through a Berkefeld or Mandler filter so as to obtain a sterile filtrate for inoculation.

Table 2 gives the results obtained with the ripe olives which caused the New York outbreak of botulism,[6] and will give some idea of this method of determining the type of B. botulinus in infected foods. The olive liquor was filtered through a Mandler filter and the inoculations shown in the table were made.

[6] Sisco, D. L.: Jour. Am. Med. Assn., 74, 1920, p. 516.

From these results it was possible to make a diagnosis of type A infection within a few hours. Ordinarily it is recommended to inoculate some mice with larger quantities, 0.5 to 1.0 c c, of the suspected filtrate, for the toxin may be present in weak concentration. The approximate strength of the antitoxin must be known so as to be certain that a sufficient amount is used to protect against the toxin injected. In the case mentioned 1 c c of antitoxin, type A, protects against about 3,000 M L D of homologous toxin; while 1 c c of antitoxin, type B, protects against about 10,000 M L D of type B toxin.

The specificity of the toxin and antitoxin of the two types, A and B, is very distinct, as I have been unable to obtain any protection against a heterologous toxin when using even massive doses of antitoxin. For example, 1,000 units of type B antitoxin (1 unit of antitoxin was arbitrarily set as the amount of antitoxin which would protect a 15 gm. mouse against 1 M L D of homologous toxin) failed to protect a mouse against 10 M L D of type A toxin, while 600 units of type A antitoxin failed to protect a mouse against 10 M L D of type B toxin. There is thus no danger in obtaining cross protection in the type determination tests when using massive doses of heterologous antitoxin. These results again emphasize the importance of using specific type antitoxin in treatment of botulinus intoxication.